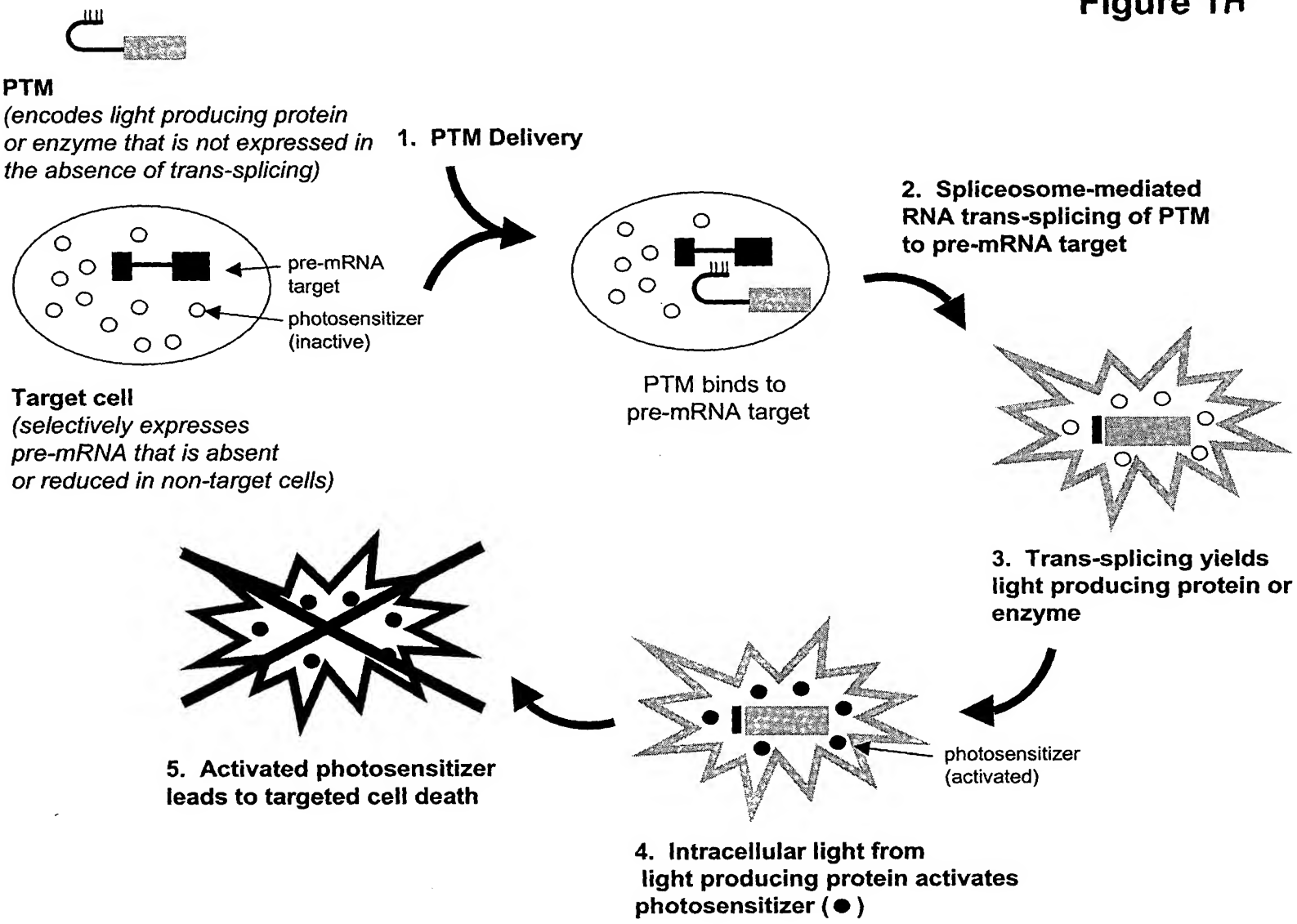
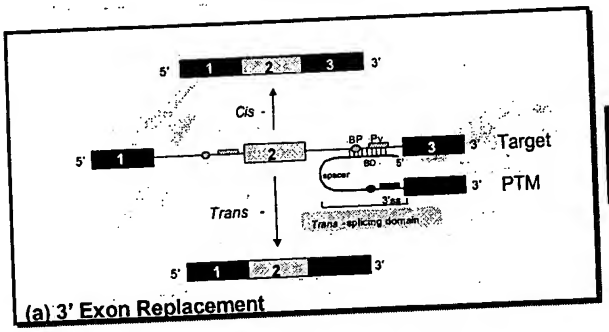
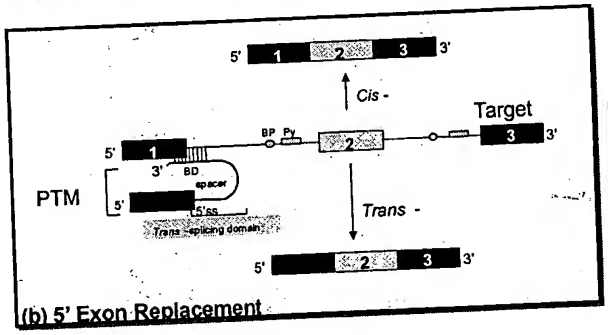
Figure 1A

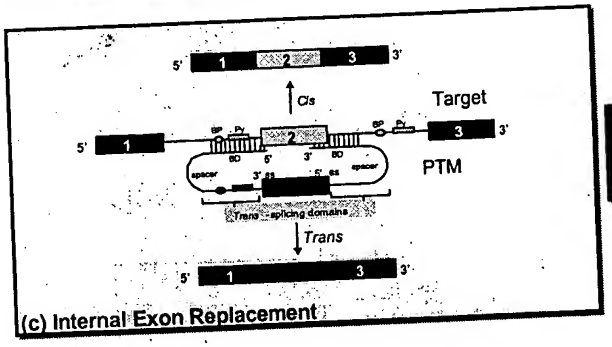

PTM
(encodes light producing protein or enzyme that is not expressed in the absence of trans-splicing)
1. PTM Delivery
pre-mRNA target
photosensitizer (inactive)
Target cell
(selectively expresses pre-mRNA that is absent or reduced in non-target cells)
PTM binds to pre-mRNA target
2. Spliceosome-mediated RNA trans-splicing of PTM to pre-mRNA target
3. Trans-splicing yields light producing protein or enzyme
photosensitizer (activated)
4. Intracellular light from light producing protein activates photosensitizer (●)
5. Activated photosensitizer leads to targeted cell death



3' Exon replacement



5' Exon replacement



Internal exon replacement

Figure 1B

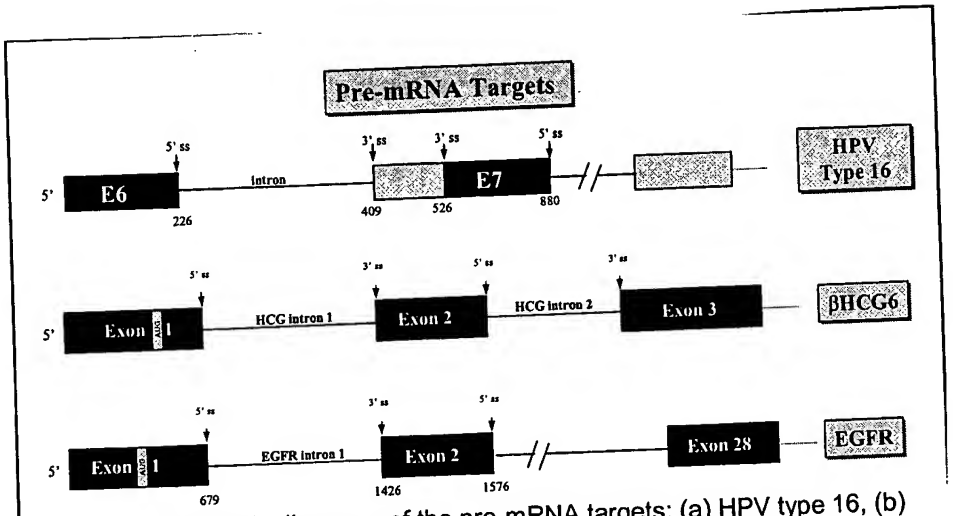


Figure 3. Schematic diagrams of the pre-mRNA targets: (a) HPV type 16, (b) β-HCG6 and (c) EGFR.

Figure 2

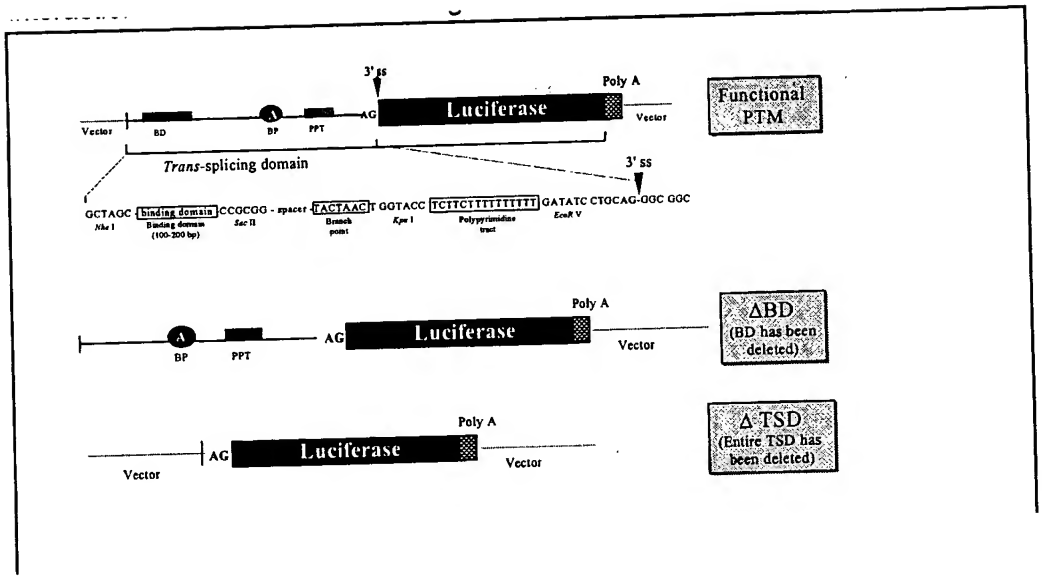

3' ss
Poly A
Luciferase
Vector
BD
BP
PPT
AG
Functional PTM
Trans-splicing domain
3' ss
GCTAGC binding domain CCGCGG - spacer - TACTAAC T GGTACC TCTTCTTTTTTTTT GATATC CTGCAG-GGC GGC
Nhe I
Binding domain (100-200 bp)
Sac II
Branch point
Kpn I
Polypyrimidine tract
EcoR V
Poly A
Luciferase
Vector
BP
PPT
AG
ΔBD (BD has been deleted)
Poly A
Luciferase
Vector
Vector
AG
Δ-TSD (Entire TSD has been deleted)

Figure 3

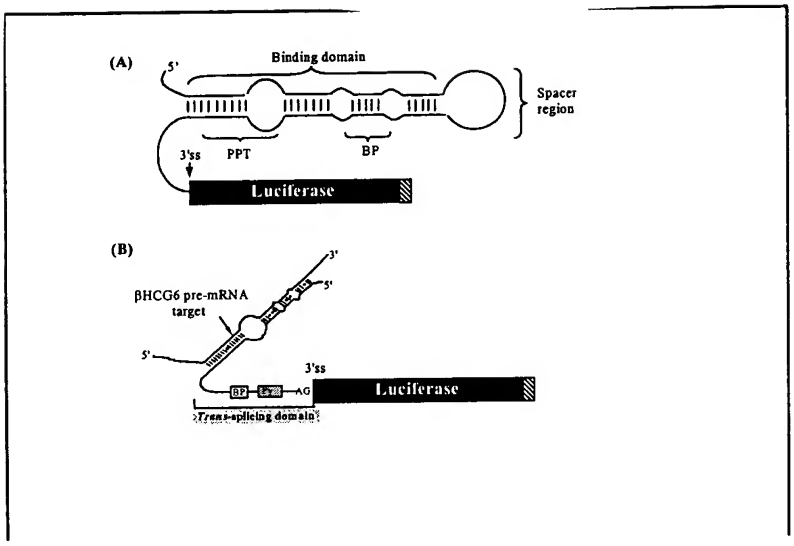

(A)
5'
Binding domain
Spacer region
3'ss
PPT
BP
Luciferase
(B)
βHCG6 pre-mRNA target
3'
5'
3'ss
BP
AG
Luciferase
Trans-splicing domain

Figure 4

R35003
(sheet 6 of 31)

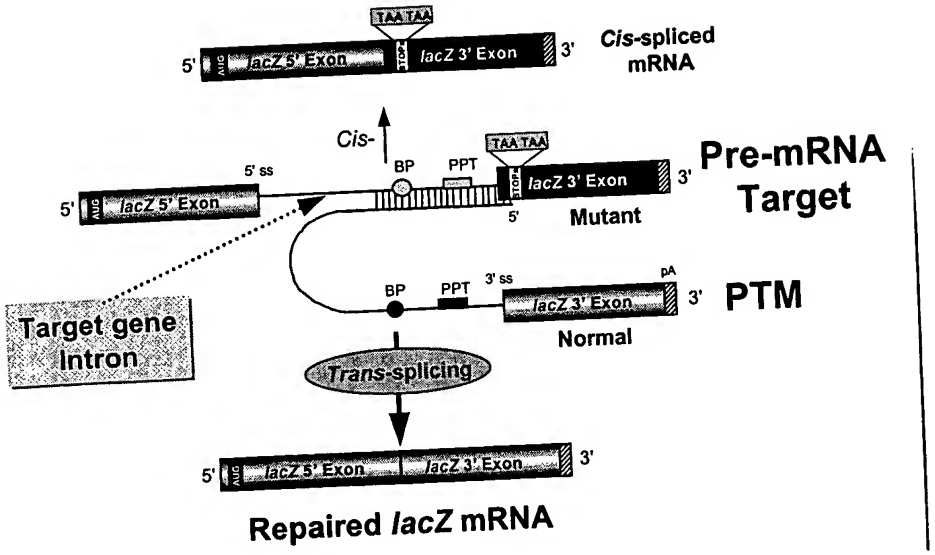
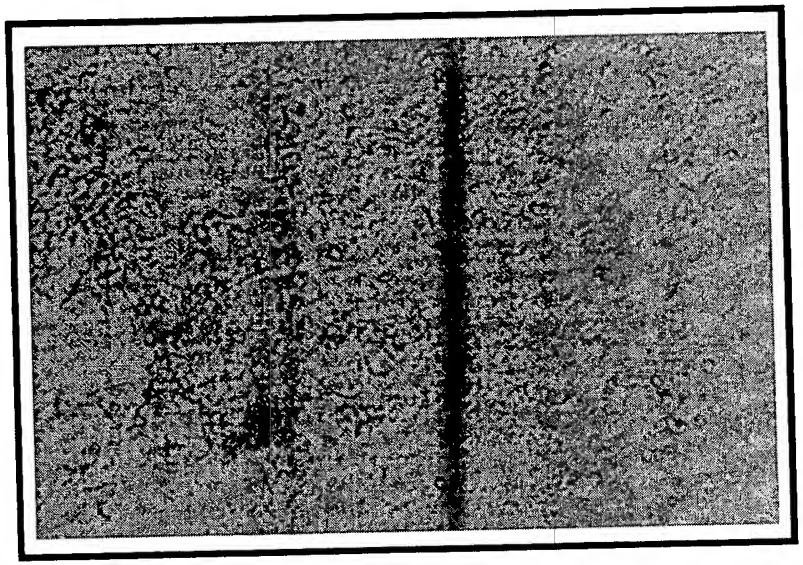


Figure 5A

Restoration of β-Gal Function

In situ **staining for β-Gal activity following co-transfection in 293T cells (unselected)**

Target alone



Target + PTM

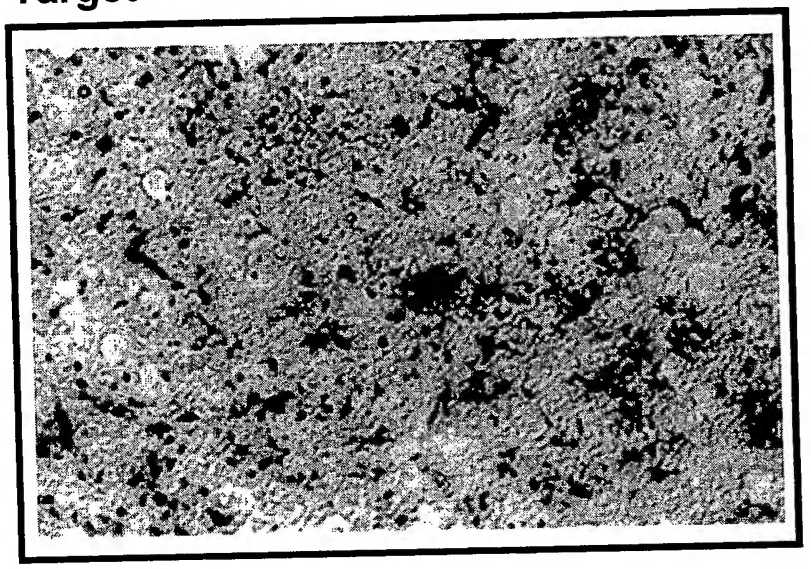


Figure 5B

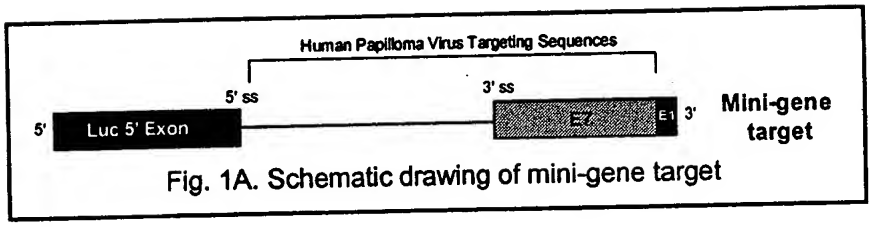


Fig. 1A. Schematic drawing of mini-gene target

Figure b

A35003
(Sheet 9 of 31)

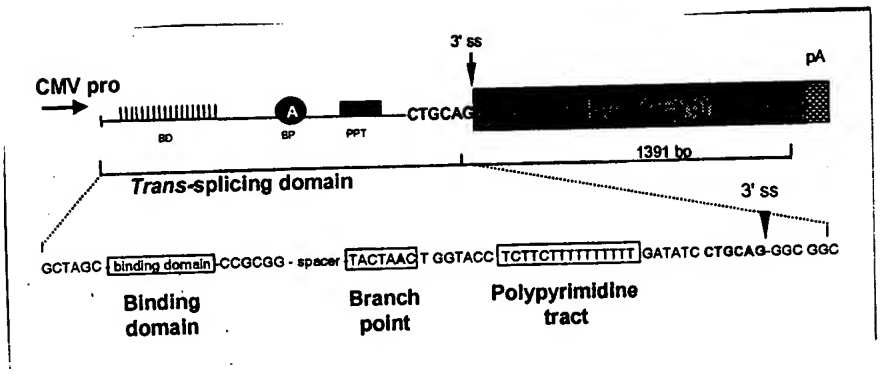


Figure 7

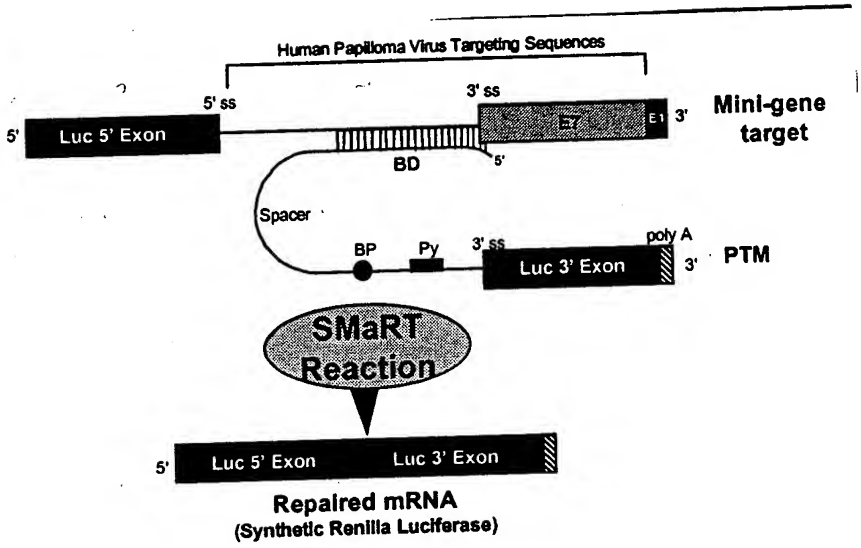

Human Papilloma Virus Targeting Sequences
5' ss
3' ss
5'
Luc 5' Exon
E7
E1
3'
Mini-gene target
BD
5'
Spacer
BP
Py
3' ss
poly A
Luc 3' Exon
3'
PTM
SMaRT Reaction
5'
Luc 5' Exon
Luc 3' Exon
Repaired mRNA
(Synthetic Renilla Luciferase)

Figure 8

A35003
(Sheet 11 of 31)

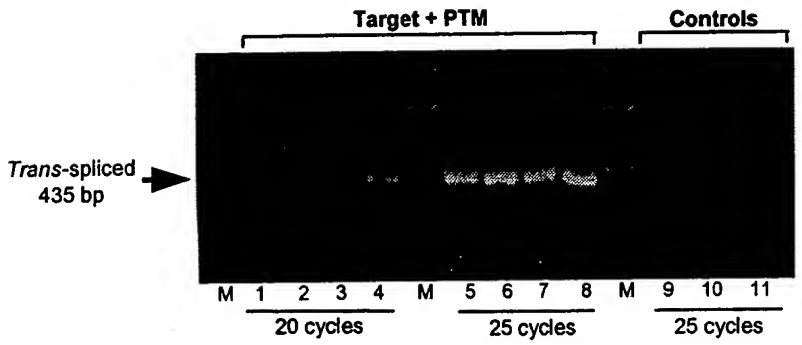


Figure 9

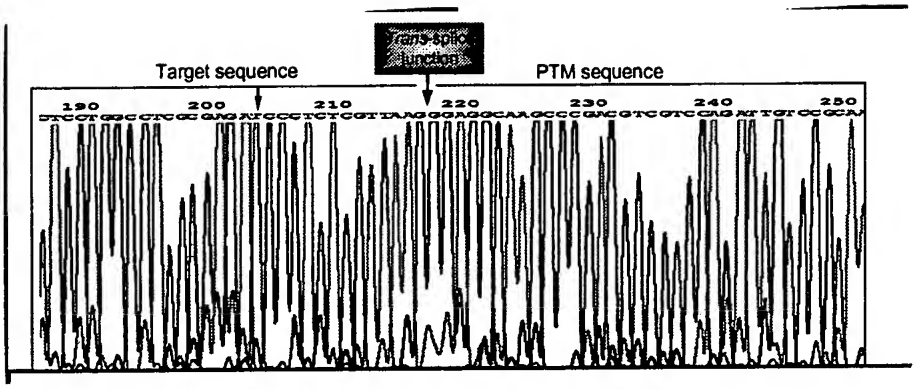

trans-splice junction
Target sequence
PTM sequence
190
200
210
220
230
240
250

Figure 10

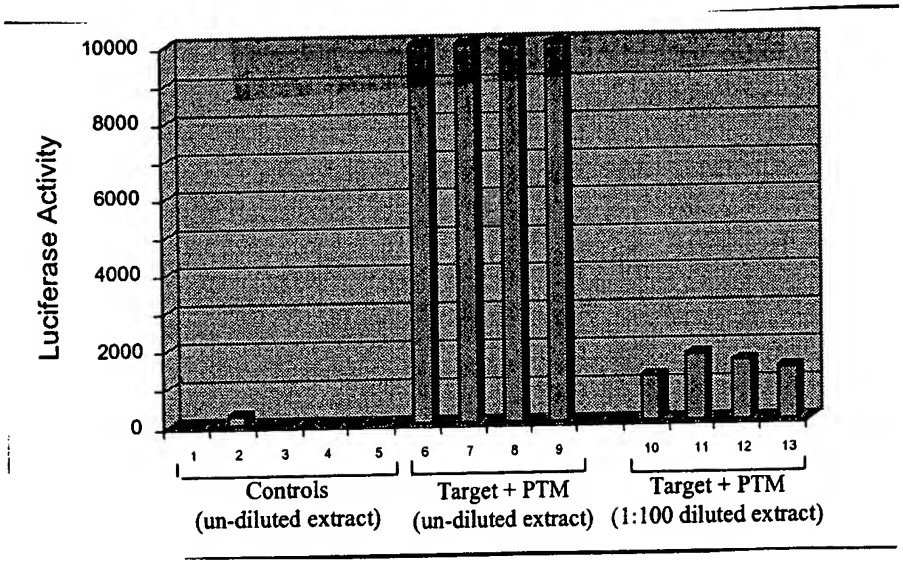

Luciferase Activity
10000
8000
6000
4000
2000
0
1
2
3
4
5
6
7
8
9
10
11
12
13
Controls
(un-diluted extract)
Target + PTM
(un-diluted extract)
Target + PTM
(1:100 diluted extract)

Figure 11

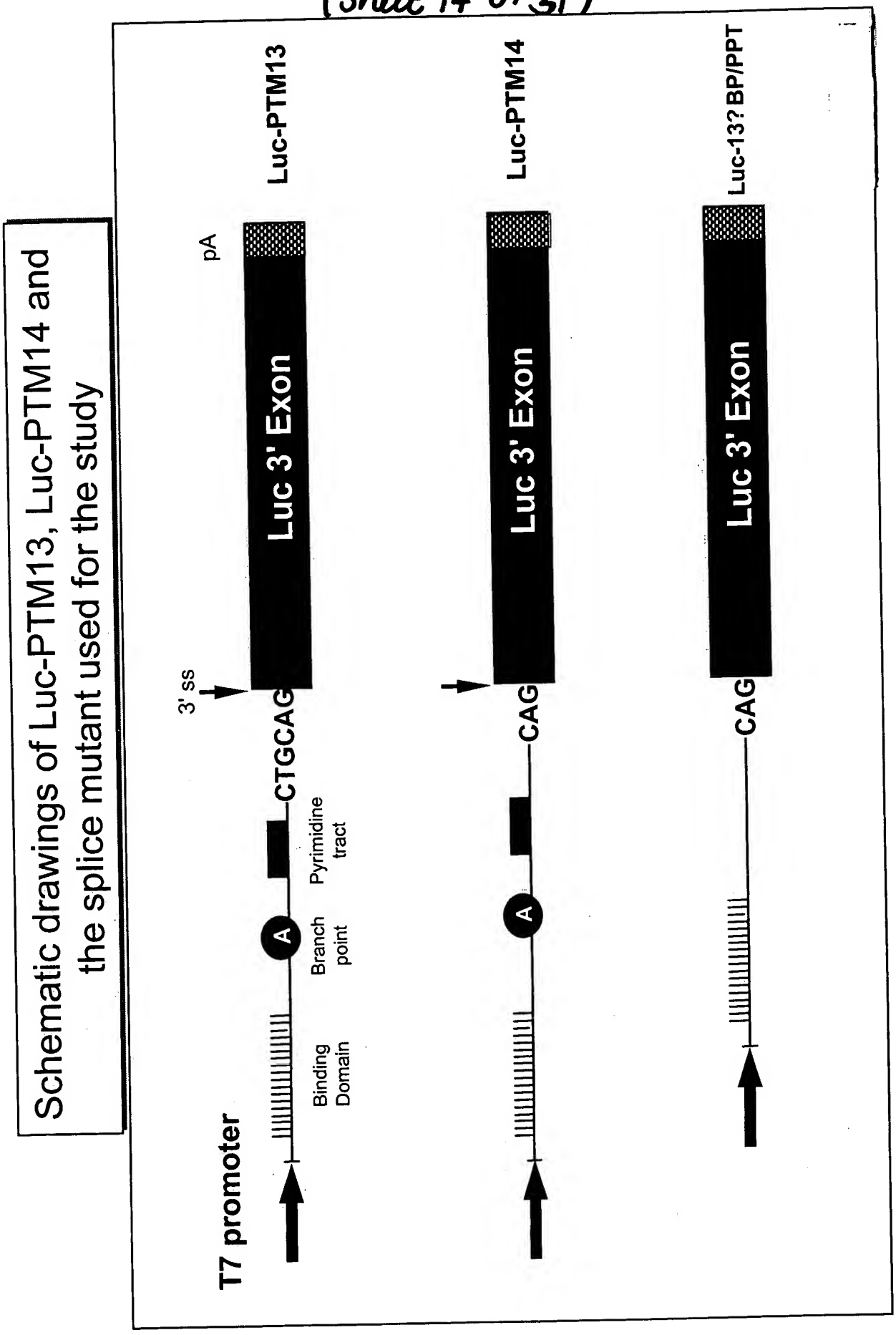

Schematic drawings of Luc-PTM13, Luc-PTM14 and the splice mutant used for the study
T7 promoter
Binding Domain
Branch point
Pyrimidine tract
3' ss
CTGCAG
pA
Luc 3' Exon
Luc-PTM13
CAG
Luc-PTM14
Luc-13? BP/PPT

Fig. 12

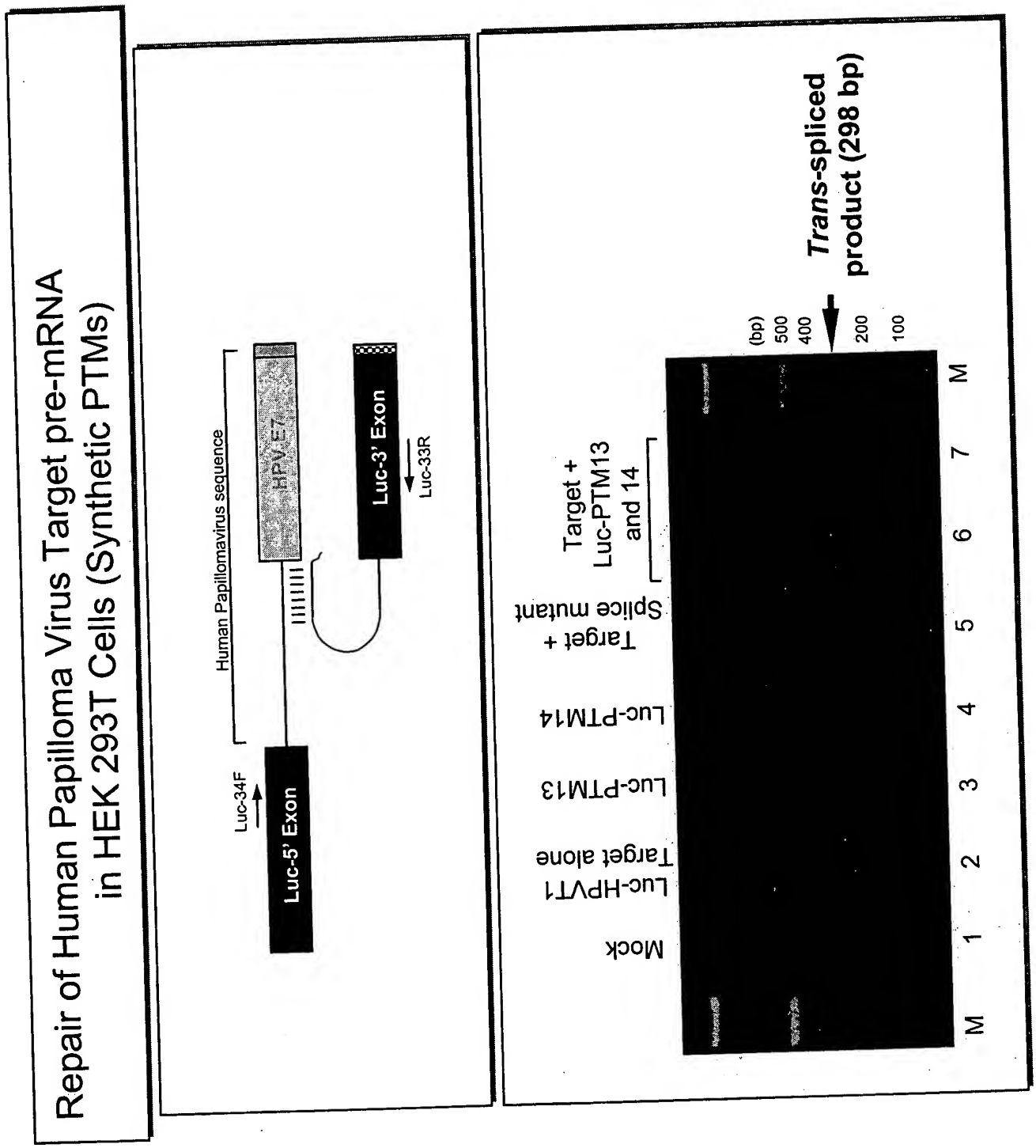
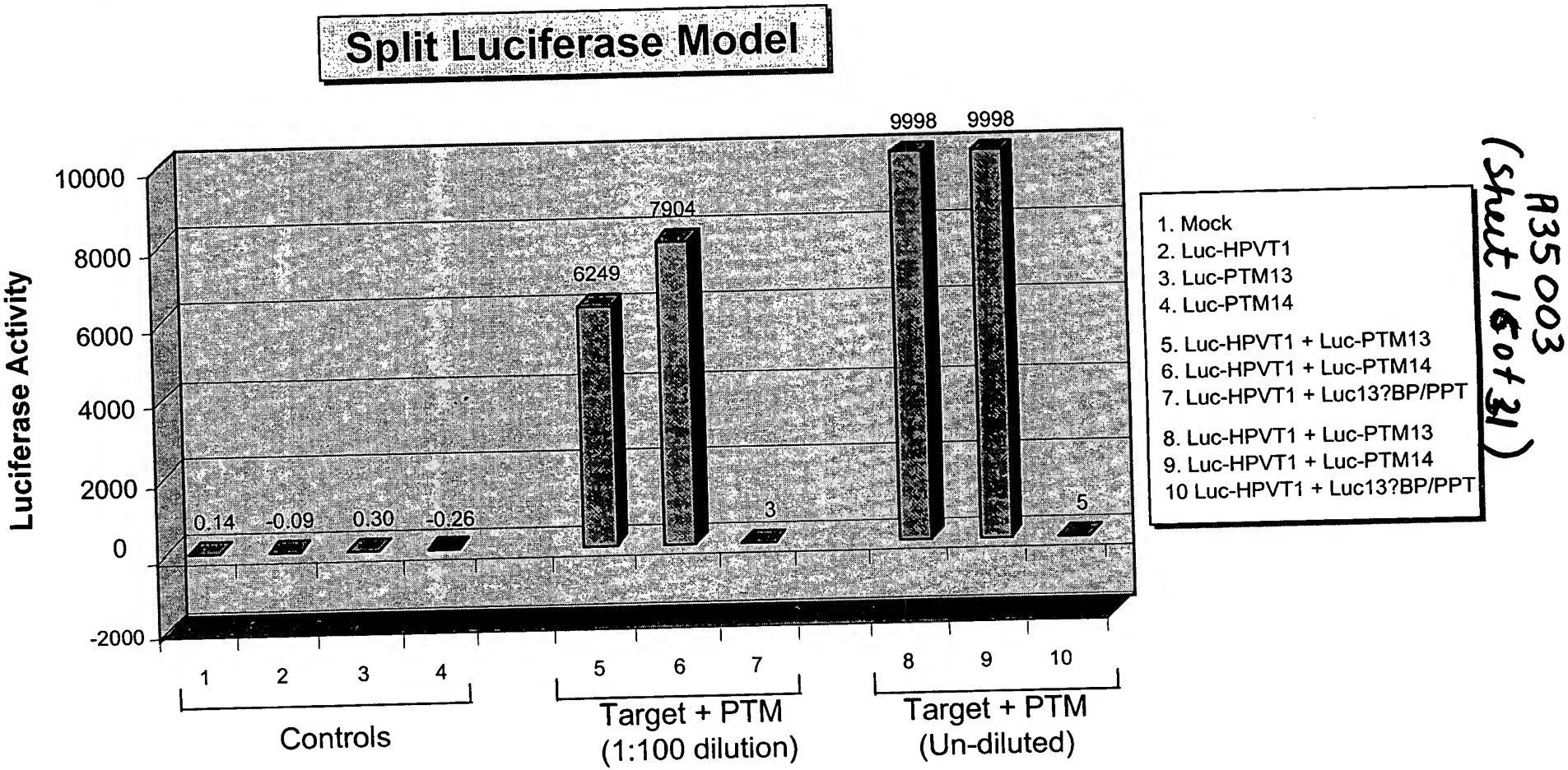

Repair of Human Papilloma Virus Target pre-mRNA in HEK 293T Cells (Synthetic PTMs)
Human Papillomavirus sequence
HPV-E7
Luc-5' Exon
Luc-34F
Luc-3' Exon
Luc-33R
Trans-spliced product (298 bp)
(bp)
500
400
200
100
M
1
2
3
4
5
6
7
M
Mock
Luc-HPVT1
Target alone
Luc-PTM13
Luc-PTM14
Target + Splice mutant
Target + Luc-PTM13 and 14

Fig. 13

Repair of Human Papilloma Virus Target pre-mRNA
Restoration of Luciferase Function in HEK 293T Cells (Synthetic PTMs)



R35003 (sheet 16 of 31)

Fig. 14

Schematic of Luciferase pre-trans-splicing molecules (PTMs)

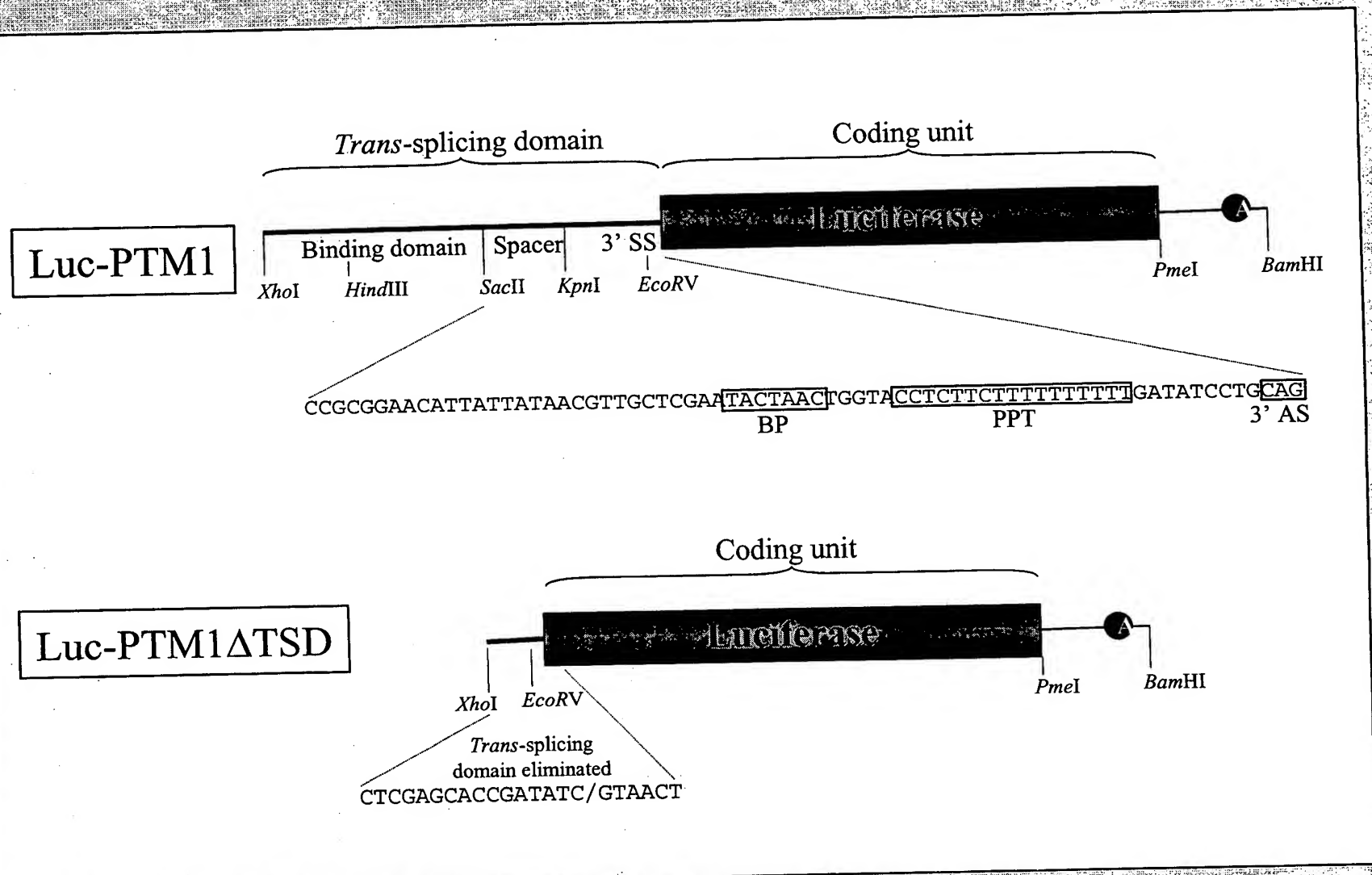
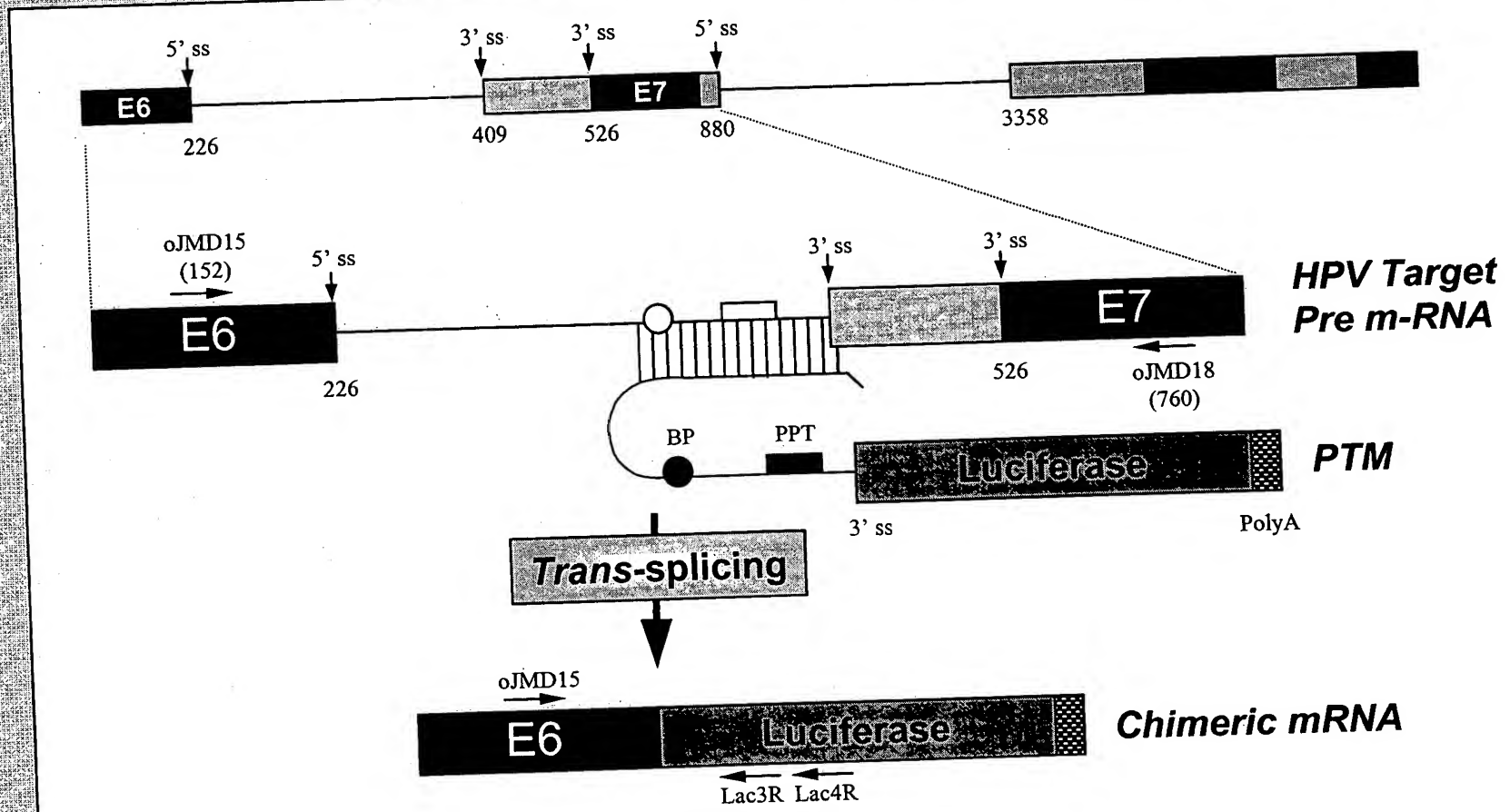


Figure 15

M35003
(Shut 17 of 31)

Strategy to Monitor the Expression of Human Papilloma Virus

Firefly Luciferase 3' exon replacement



JMD15+Lac4R = 565 bp
JMD15 + Lac3R= 398 bp

Figure 16

B5003 (Sht 18 of 31)

Luciferase data (with and without target).

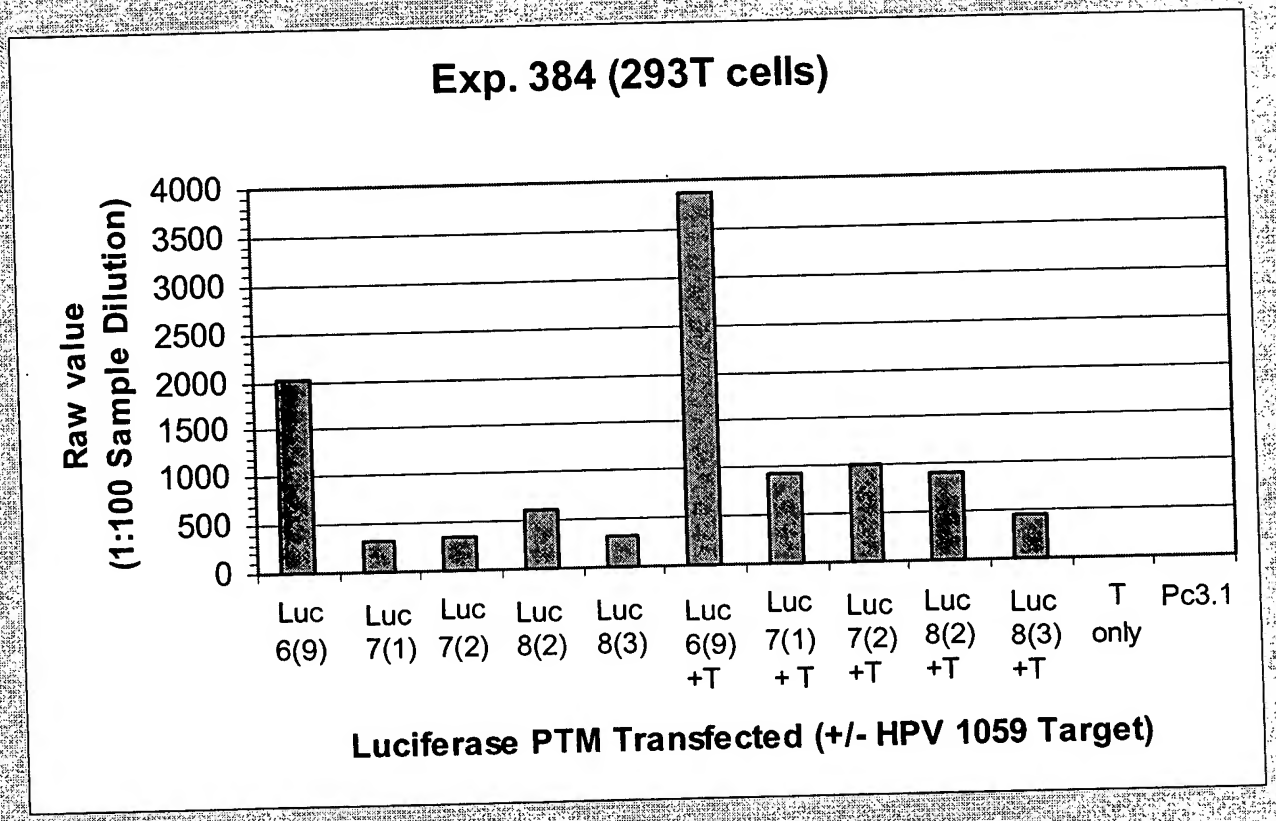
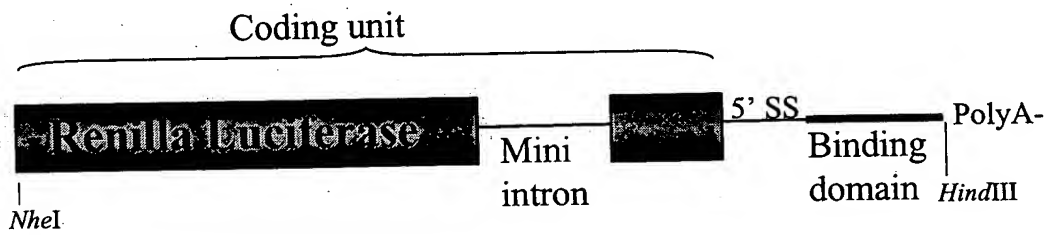


Figure 17

Schematic of Renilla Luciferase pre-trans-splicing molecule (PTM)



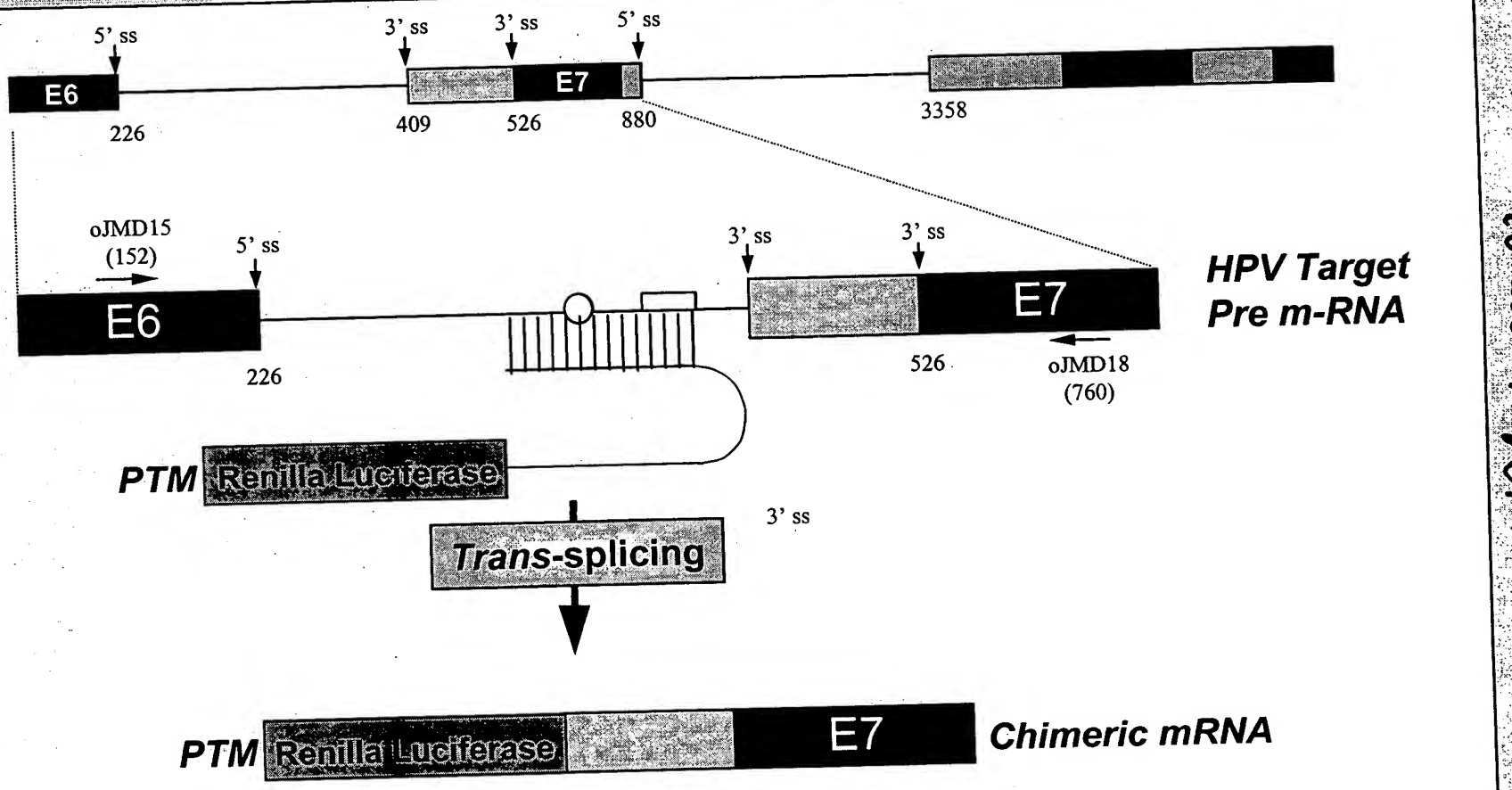
Ren-PTM1

Figure 18

Figure 19

SMaRT Strategy to Monitor the Expression of Human Papilloma Virus

Renilla 5' exon replacement



R35003 (Ghut 21 of 31)

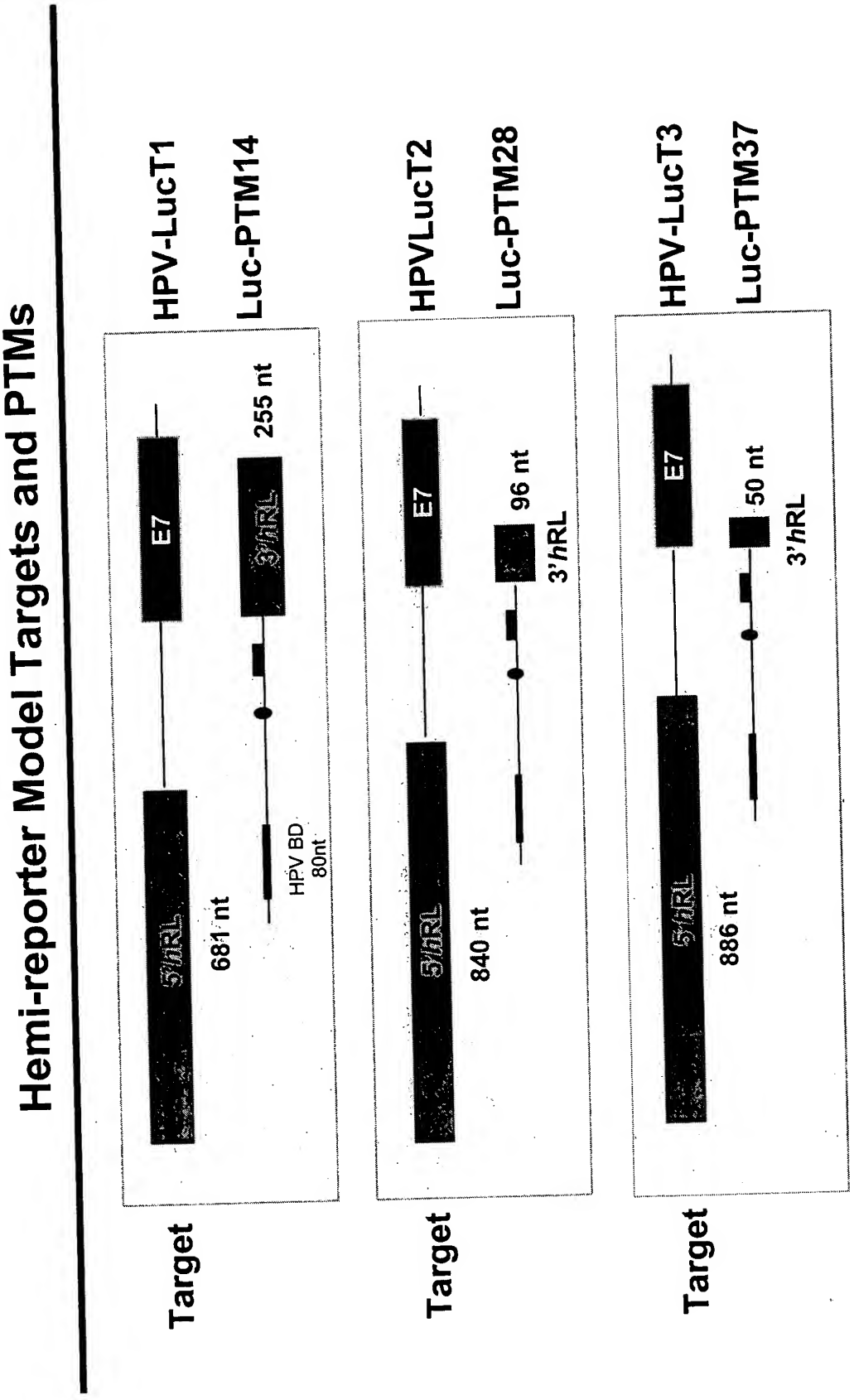
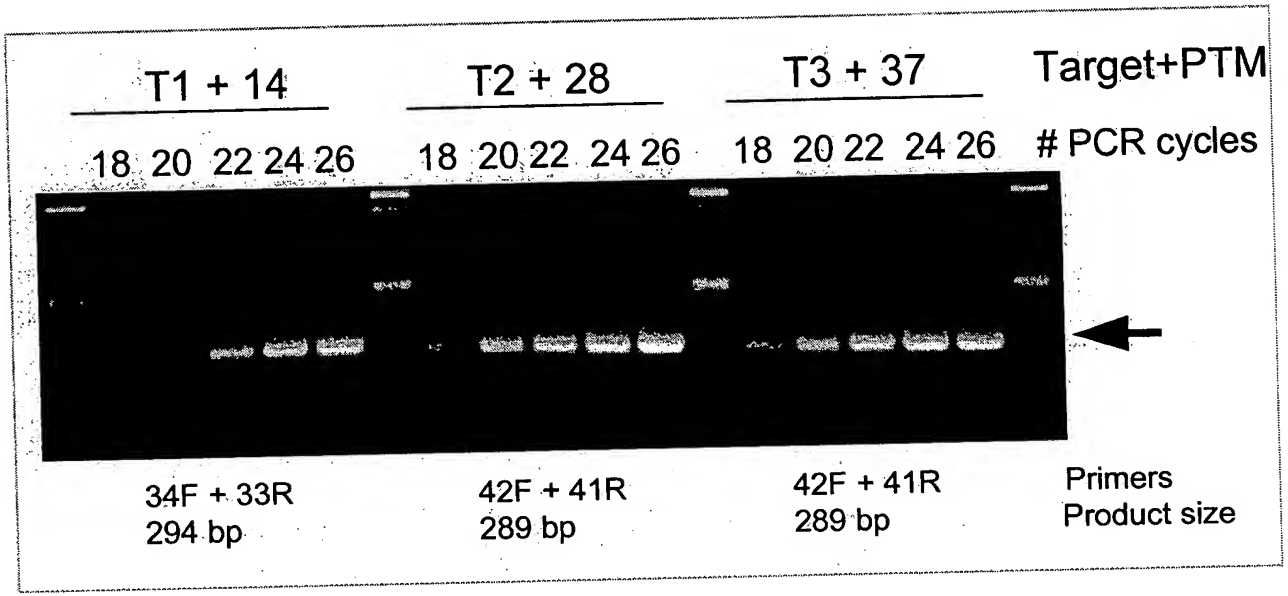
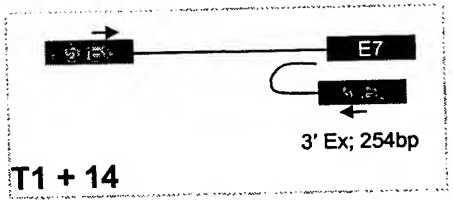

Hemi-reporter Model Targets and PTMs
Target
HPV-LucT1
Luc-PTM14
E7
5'hRL
681 nt
255 nt
3'hRL
HPV BD
80nt
Target
HPVLucT2
Luc-PTM28
E7
3'hRL
840 nt
96 nt
3'hRL
Target
HPV-LucT3
Luc-PTM37
E7
5'hRL
886 nt
50 nt
3'hRL

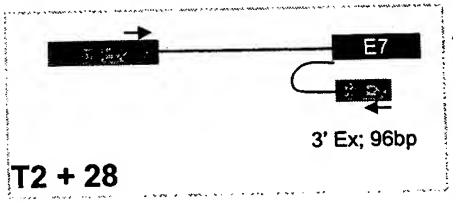
Figure 20

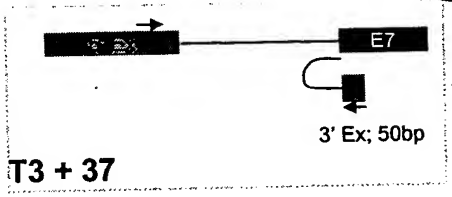
Trans-splicing efficiency at the RNA level

(semi-quantitative RT-PCR)







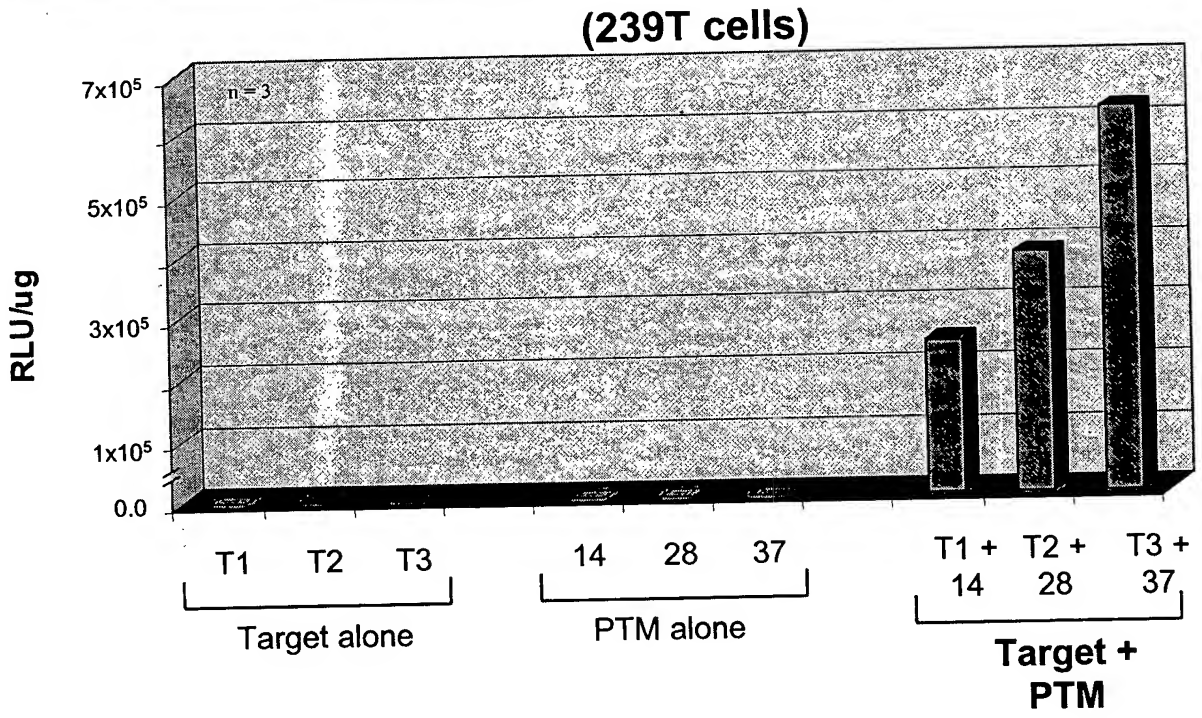


H35003 (shut 23 of 31)

Figure 21

INTRONN

Efficient & accurate *trans*-splicing restores luciferase function in 293T cells



A35003
(shut 24 of 31)

Figure 22

Imaging of gene expression using full-length reporter PTM

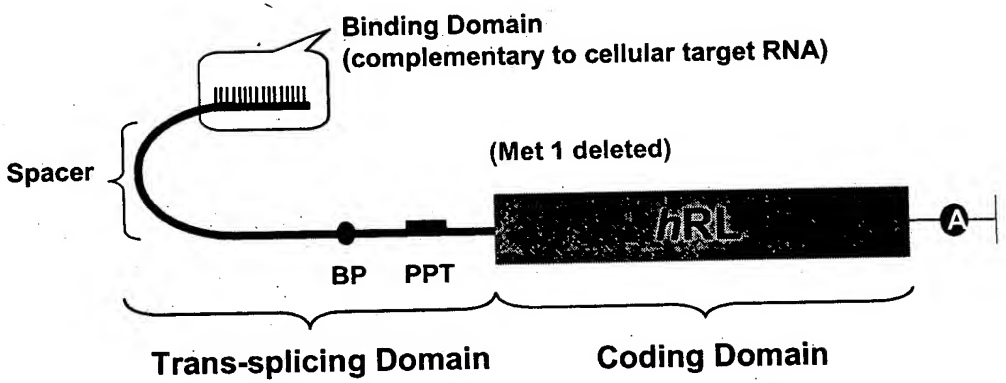


Figure 23A

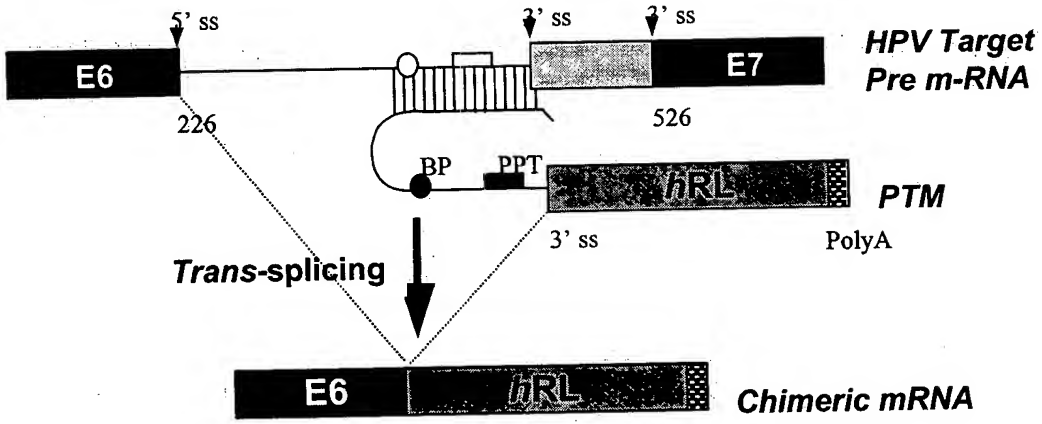


Figure 23B

Trans-splicing mediated mRNA repair and restoration of *h*Renilla Luciferase activity in 293T cells

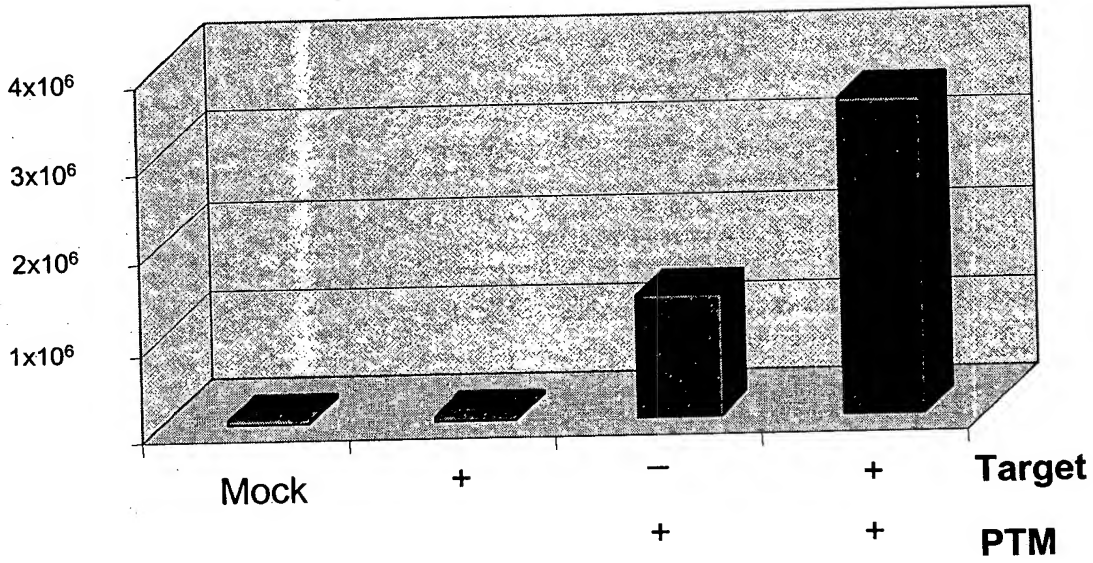
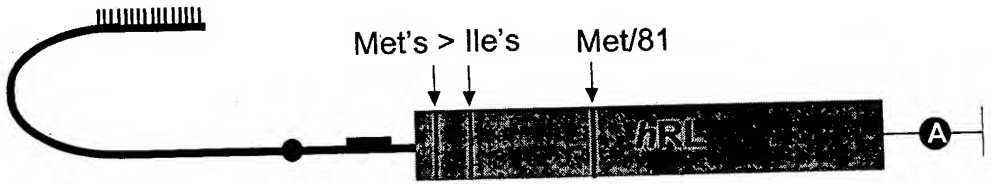


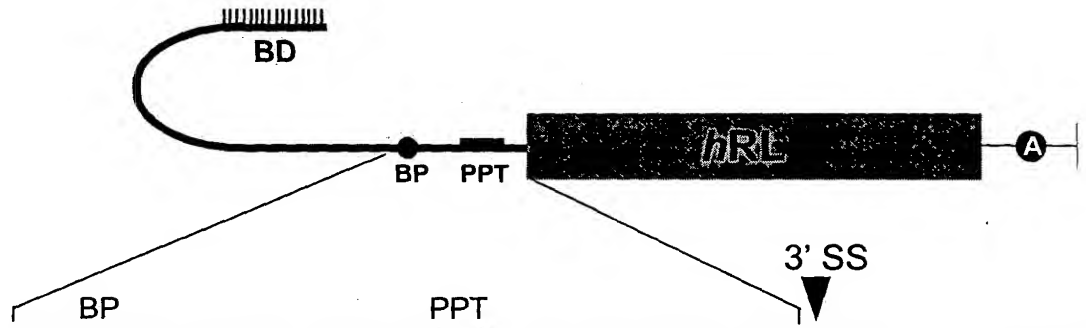
Figure 24



Structure of a full-length imaging PTM (Luc-PTM38)

Figure 25A

Luciferase mutant PTM



A35003
(Shut 28 of 31)

Figure 25B

INTRONN

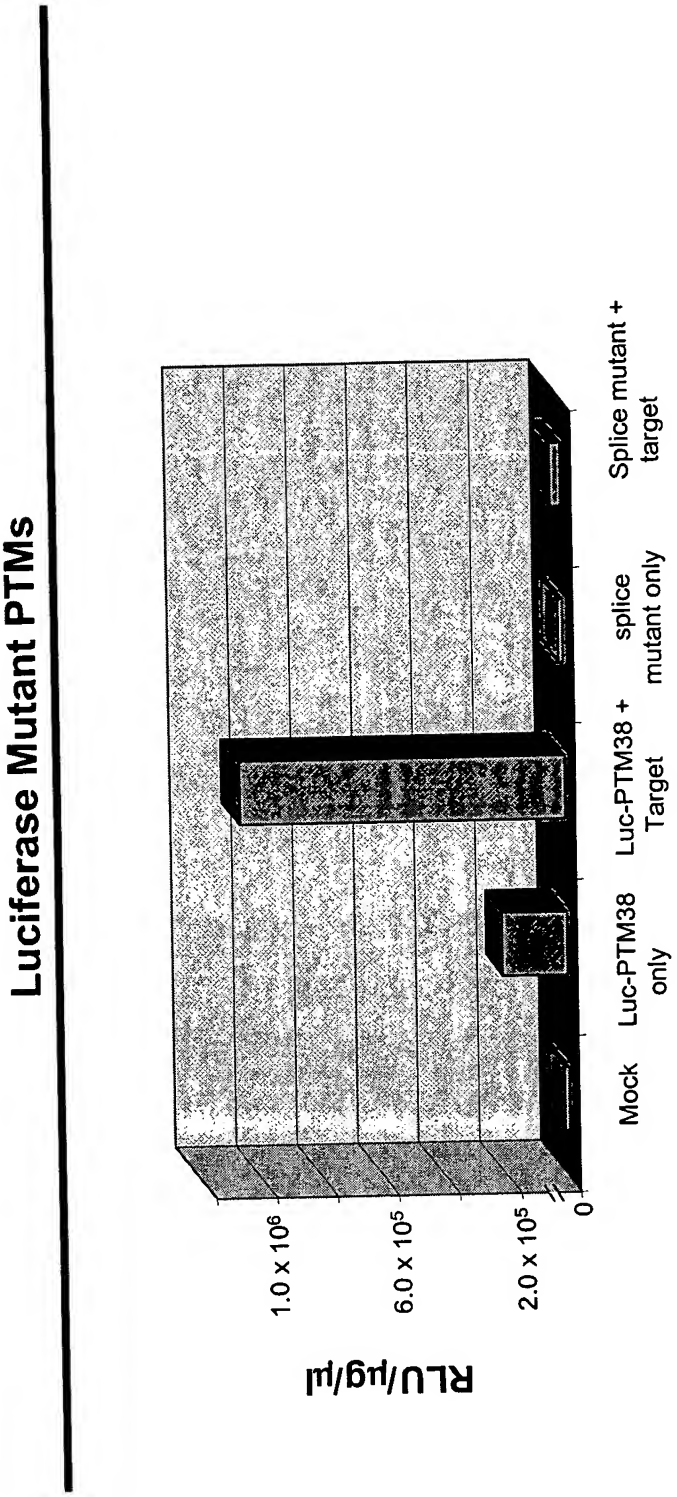
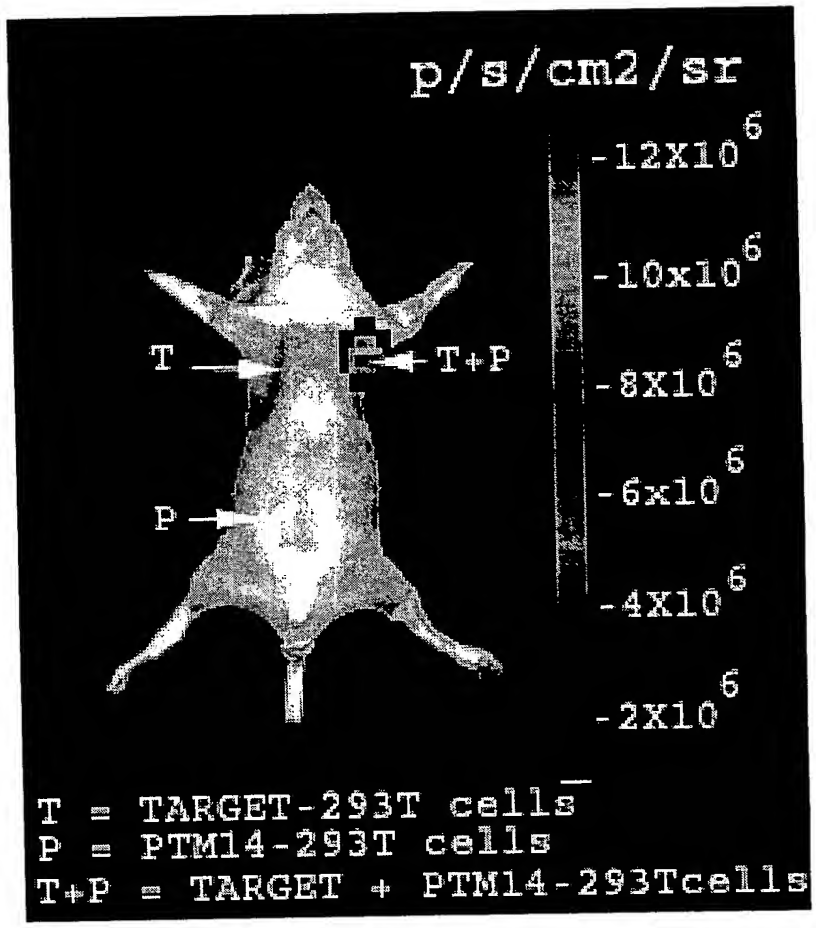
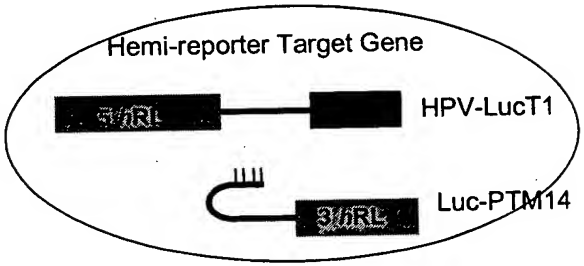

Luciferase Mutant PTMs
RLU/µg/µl
1.0 x 10^6
6.0 x 10^5
2.0 x 10^5
0
Mock
Luc-PTM38 only
Luc-PTM38 + Target
splice mutant only
Splice mutant + target

Figure 26

Imaging Gene Expression (*In vivo*)





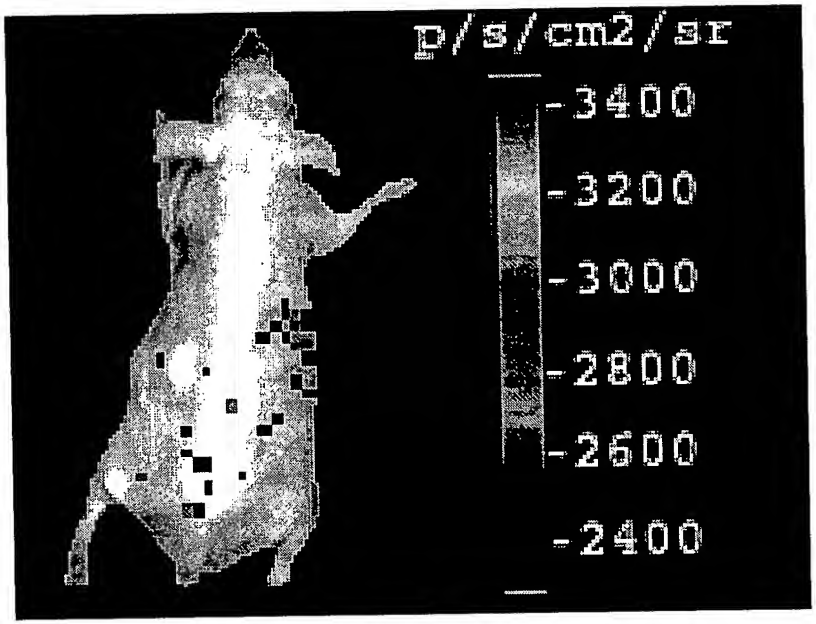
Targ t + PTM used for the above study

Figure 27

INTRONN

A 35003
(Shut 30 of 31)

Imaging Gene Expression In Vivo following IV PTM delivery



HPV-LucT1
+
Luc-PTM14

A35003
(Amt 31 of 31)

Figure 28